Personal Care Products Council
Committed to Safety, Quality & Innovation

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Dear Dr. White:

The following comments are submitted on behalf of the Personal Care Products Council[1] in response to the National Toxicology Program (NTP) Board of Scientific Counselors Technical Report Subcommittee's review of "*NTP Technical Report on the Photococarcinogenesis Study of Retinoic Acid and Retinyl Palmitate in SKH-1 Mice", (*TR568*)* scheduled to be peer reviewed on January 26, 2011.

NTP BSC Technical Report Review Panel Charge

Retinyl palmitate is approved by U.S. Food and Drug Administration (FDA) as a food GRAS nutrient and as an over-the-counter (OTC) and prescription drug. To achieve premarket approval, FDA, which is the U.S. regulatory authority for retinyl palmitate when used as a drug, in foods and in cosmetics, required extensive and rigorous pre-market testing. It is important that NTP Technical Report (TR) panels recognize that NTP is not a regulatory authority. We were therefore encouraged to note that NTP's charge to the panel is focused and crisp: **(1) peer review the scientific and technical elements of the study and its presentation; (2) determine whether the study's experimental design and conduct support the NTP's conclusions regarding the carcinogenic activity of the substance tested.**

Retinyl Palmitate Nomination

In November, 2000, the FDA's Center for Food Safety and Applied Nutrition (CFSAN) nominated Retinyl Palmitate (RP) to the National Toxicology Program requesting *"– a*

[1] Based in Washington, D.C., the Council is the leading national trade association representing the $250 billion global cosmetic and personal care products industry. Founded in 1894, the Council's more than 600 member companies manufacture, distribute, and supply the vast majority of finished personal care products marketed in the United States. As the makers of a diverse range of products that millions of consumers rely on everyday, from sunscreens, toothpaste, and shampoo to moisturizer, lipstick, and fragrance, member companies are global leaders committed to product safety, quality, and innovation. The Council was previously known as the Cosmetic, Toiletry, and Fragrance Association (CTFA).

photocarcinogenesis study of retinyl palmitate, under conditions relevant to the use of retinyl palmitate in cosmetics" – and -- "mechanistic studies to establish the relevance of the results obtained in the selected animal model". In the draft TR 568 report, made available in December, 2010**,** the nomination rational and testing request states "--- *for phototoxicity and photocarcinogenicity testing based on the increasingly widespread use of this compound in cosmetic retail products for use on sun-exposed skin, the biochemical and histological cutaneous alterations elicited by RP, and the association between topical application of retinoids and enhancement of photocarcinogenesis".* While the term "sunscreen" is not mentioned, we believe it may be implied. **The NTP RP protocol was not properly constructed to test sunscreens or sun blockers containing RP**.

Time Line

We understand the FDA nominated RP to the NTP in 2000, that the one (1) year photo-cocarcinogenesis study was begun in 2003 and that the on-site pathology was not completed until mid-2006. While it is recognized that delays can occur in any study, we question the two-year delay in pathology completion, since the 1% and 2% RP animals did not have pathology performed, and especially the four-year delay from pathology completion to the availability of the draft RP TR in December 2010. **Because of the reported study flaws in the TR 568 report, we wonder if NTP had concerns about the adequacy of the study or ever considered not bringing the study forward.**

Protocol Design

In the standard UVR SKH-1 protocol designed by Forbes, animals receive test agent followed by UVR exposures on Monday, Wednesday and Friday and receive UVR exposures followed by test agent on Tuesday and Thursday, a routine followed for 40 weeks with an additional 15 week no dose/no UVR monitoring; endpoints are typically time to lesion formation (specified size) and/or lesion multiplicity. The exposure protocol for this study was different in that treatment with UVR was in the morning 5 days a week and treatment with test agents was in the afternoon 5 days a week then, after 40 weeks of treatment, the mice were held without treatment for additional 12 weeks prior to sacrifice. The exposure protocol for the TR 568 report was selected to *"mimic human use where people are exposed to sunlight during the afternoon then use the retinoid-containing creams at night"*. **We wonder how the change in the exposure protocol from the widely accepted Forbes standard protocol could have influenced the outcome of these studies.**

Reasons for Removal

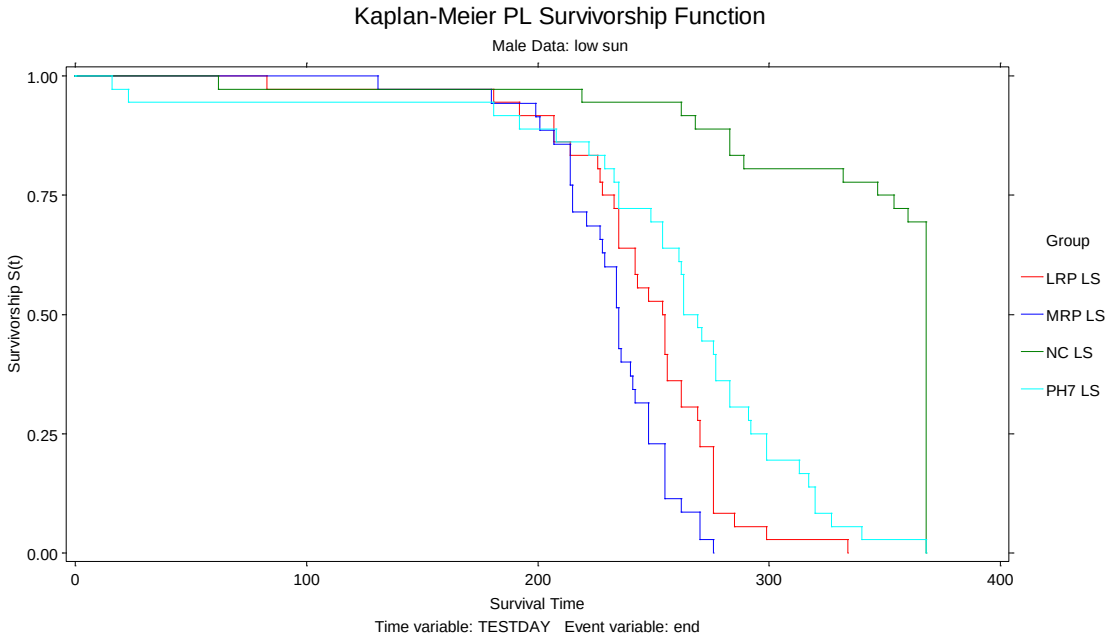
Tables 4 and 5 in TR 568 show that in groups with control cream and RP, the main reason for animals exiting the study was Skin Lesion ≥ 10 mm. However, in the Preliminary Pathology Tables presented on the NTP web site the cause for removal was listed as "harvest." We believe that removal criteria other than tumors ≥10 mm may have been used when determining whether or not to remove animals under the "harvest" terminology (e.g. due to severe toxicity). It would benefit the reader if the Standard Operating Procedure (SOP) for animal removal were included in the appendices of TR568 since the removals were considered to be non-censored animals (known lifetime) and the vast majority of animals exposed to sunlight/cream or sunlight/cream + RP were in the "removed" category. Therefore the criteria for selecting

animals for removal from this experiment were considered non-censored in spite of the fact that many were removed due to toxicity (and thus should have been classified as censored animals).

Note that the issue of censoring is of more than academic interest. For example, one might have comparable numbers of tumors in each of two dose groups but if animals were removed sooner in the first group, it would have a higher tumor rate than the second because the first group would have fewer animal-years. At a more general level, the report notes that the survival analyses presented are in fact survival-removal analyses. So what exactly does removal mean? A full understanding of the data in this study must be accompanied by a detailed discussion of the removal criteria, and the reader would also be assisted by a discussion of the relative numbers of animals removed by removal criterion.

Statistical Analysis and Confounders

Our overall impression is that the statistical analyses applied in TR 568 are appropriate, and that the signals that the test system is generating may appear reasonable to the reader not familiar with the nuances of photo-cocarcinogenesis bioassays. However, we note that the difference between the response of control cream plus UVR compared to UVR only is unacceptably dramatic (see Example 1 this document). We also note that 1% and 2% RP formulations appear toxic even in the absence of UVR (Figures 8 and 9) and assume that this is the reason that neither 1% nor 2% RP animals appear in the pathology evaluations. **To us, this should have resulted in study termination.**



Example 1 *Time on Test (TOT) Data* *Male survival with Low-Sun treatment (6.75 mJ.CIE/cm2) for No Cream (NC - Green); Control Cream (PH7 - Turquoise); Low RP (LRP - Red); and Mid RP (MRP - Blue). Note the magnitude of the shift to the left for the Control Cream (Turquoise line) compared to the No Cream group (Green Line).* **Since the shift to the left is dramatic and unacceptable – can this really be an adequate study?**

A first concern is that there is no way to estimate the effects of RP independent from the effects of the control cream which indeed is a major problem. What would be the effects, if any, of RP administered in a control cream that did not by itself act as a promoter? One can only speculate.

A second issue is the test system itself. TR568 says that 1% and 2% RP levels caused severe skin irritation requiring animal removal, even in the absence of exposure to UVR. However, those levels have reportedly been used without such irritation in other published peer-reviewed studies. So are the 1% and 2% RP levels toxic because too high of a dose was selected, because of a property of the SKH-1 mice used in this study, or because of an effect of the interaction of RP with a component of the control cream, such as diisopropyl adipate? Again, one can only speculate.

Third, we believe it is inappropriate to use time to tumor formation and/or tumor multiplicity data from animals that exhibit toxicity and 1) were removed from the experiment early, and 2) were excluded from pathology examination (1% and 2% RP animals).

Finally, no amount of statistical sophistication or manipulation can legitimately estimate main effects in the presence of large interactions. For example, the Cox Hazard Ratios between cream and various levels of RP do not represent independent RP effects. Rather they represent the effect of the cream, the effect of RP, and the effect of the unknown but also possibly they represent large interaction between the cream and RP. We believe it is simply irresponsible to attempt to present such analyses without caveats concerning the fact that the degree to which such differences exist is unknown and in fact cannot be estimated with the available data.

Control Cream with diisopropyl adipate

A control vehicle must be known not to enhance or prevent a particular biological event; it is only a carrier of the test agent or used to simulate a particular manipulation of the test animal. If it is noted that the control vehicle elicits the same biological response that is to be measured in a study, then reasonable scientists would consider the experiment flawed and the study would be repeated using a non-reactive control vehicle or abandoned.

For this particular study, it is difficult to imagine how, 1) once it was noted that control cream animals were developing comparable numbers of tumors to the test agent animals at the same UVR dosage and/or 2) noted that animals were experiencing severe toxicity reactions requiring removal as “Harvest” (preliminary NTP Pathology Tables), that this study was allowed to proceed for the entire one (1) year duration of the experiment. Indeed, the fact that the 1% and 2% RP dosed animals were in such poor condition as to preclude pathological examination is a strong statement that this experiment was flawed and should have been terminated.

Topically applied vehicle control formulations may include water, emollients, moisturizers, ointments, creams, salves and balms. It is known that, depending on the formulation mixture, all may increase or decrease test agent absorption, change the

optical properties such that UVR penetration is enhanced or reduced or support chemical reactions between the test agent and a control formulation component. This is why it is important to test the vehicle control formulation independently to assure it does not enhance the biological event that the test is measuring. This study suffers from that oversight.

NTP has conducted many properly designed, well managed and accurately reported hazard identification studies over the years that have contributed to public health and found utility by the regulatory community. Unfortunately, for reasons discussed above, the TR 568 study does not measure up to NTP standards. **Therefore, we believe that the only reasonable call that NTP can support for TR 568 is: Inadequate Study of Carcinogenic Activity.**

UVA / UVB Studies

In a separate experiment the NTP tested RP (1.0%) in female SKH-1 mice in the presence and absence of UVA or UVB irradiation. This study utilized the same control cream and thus suffers from the same experimental flaws noted with the UVR study. We believe the results from the UVA / UVB study can only be viewed as "observational" and certainly **cannot be utilized in any capacity to support the NTP call for the one (1) year photo-cocarcinogenesis study.**

Initiation/Promotion/Progression UVR/SKH-1 Animal Model

The SKH-1 / UVR protocol design is a (x) staged initiation-promotion-progression design model. A slope shift to the left could be due to (1) photo-activated production to a bioactive chemical, (2) modulation of UVR-induced genotoxicity, (3) simple enhanced promotion of UVR-initiated cells, (4) test agent acting additively/synergistically with UVR, (5) simple phototoxicity, (6) immune suppression, (7) interaction between control cream and test agent,(8) altered apoptosis, (9) a combination of the above, or (10) other unknown mechanisms. The variability in outcome when testing various substances, including the retinoids, using this animal model is quite large, as even noted in this TR. Study results are more often a consequence of protocol design, test agent purity, exposure times, test agent and UVR application sequence and the type of control vehicle utilized.

Moreover, UVR is, by itself, the initiator and the promoter (it is a "complete" carcinogen) as nicely demonstrated in the UVR dose curves published in this TR; this by itself is a confounder when attempting to interpret study outcomes. Clearly, extrapolation concerns must also exist when considering animal vs. human differences in test agent response, UVR response and/or response to the combination of test agent/UVR. So the question for the FDA becomes: **how does one even begin to understand what a slope shift to the left means in the presence of a test agent and UVR, in a regulatory framework? That is, how does FDA measure the "risk to human health" from such animal studies? Furthermore, can the FDA really regulate an animal photo-cocarcinogen "promoter"?**

Given those questions, we note with interest that the FDA Center for Drug Evaluation and Research (CDER) has recently published, "Guidance for Industry M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing

Authorization for Pharmaceuticals" which in Note 6, states: **"Testing for photocarcinogenicity in rodents using currently available models (e.g., hairless rodent) is not considered useful in support of pharmaceutical development and generally is not recommended**. We wonder if this same policy is also embraced by CFSAN and other FDA product centers.

Summary

There was what we believe an unusual 11-year delay from FDA nomination to NTP reporting the results from this one (1) year photo-cocarcinogenesis study (2000 – 2011) and speculate that the delay may have been driven by NTP questioning the adequacy of the study and debating the merits of bringing this study forward for a public peer review.

The NTP used a protocol design different from the accepted Forbes design which was, we believe, not adequately justified in TR 568 and furthermore was an untested design at the beginning of the RP photo-cocarcinogenesis study. The impact on the outcome of the TR 568 is uncertain.

The UVA and UVB studies suffer from the same confounder's that the UVR study does (active control cream) and can only be viewed as observational in nature and should not be used in any manner in supporting the call for TR 568.

No reasonable scientist would have continued a study so obviously flawed by the presence of a reactive control cream that alone dramatically changed the slope of the response. Moreover, the obvious toxic response to RP dosing in the presence and absence of UVR was another reason to terminate the study.

It is impossible to determine the independent action of RP on the development of skin tumors or tumor multiplicity. Additionally, it is difficult to imagine how any U.S or international regulatory body could use such data in a risk assessment or for formulating any reasonable risk management decision.

Finally, the only reasonable call that the NTP can support for this study is: **Inadequate Study of Carcinogenic Activity.**

Sincerely,

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Science

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